Identification of novel activators of two-pore domain potassium (K2P) channels

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OVERVIEW
• Two-pore domain potassium channels (K2P) carry background (or leak) potassium current
• Primarily act to maintain resting membrane potential.
• K2P channels are characterised by their four transmembrane domain, two-pore topology
• Genetic and functional evidence points to a role in multiple pathophysologies, including pain and migraine
• K2P activators have proven difficult to modulate with small molecules and there is a lack of useful specific pharmacological tools
• This has limited the interrogation of their precise physiological function and efforts to generate K2P-based therapeutics
• LifeArc developed a novel system to identify K2P activators, with the aim of providing tools for research and ultimately novel therapeutics

IDENTIFYING ACTIVATORS Requires Bespoke Assays/Reagents
• Identification of activators is highly dependent on the use of appropriately designed and specifically optimized assay reagents
• LifeArc developed cell-based functional assays and translational screening cascades for identification of K2P activator hits
• BacMam® allows the precise titration of expression of the gene of interest
• This enabled generation of cell systems in which we were able to intricately and robustly select a level of K2P expression, in functional assays, optimized for the identification of channel activators

ASSAY PROCESS AND METHODS
• Frozen U-2 OS cells used for all assays
• Cells transduced with K2P BacMam
• Cells plated into 384W plates and incubated overnight
• Plates read on FLIPE, Thallium addition on-line
• Compounds added to buffer using ECHO, transferred to cells on BROMEK
• Media removed and replaced with dye (Molecular Devices Potassium kit)
• Methodology designed to maximise flexibility and throughput
• Allows screening of multiple K2P channels simultaneously

SCREENING FOR K2P ACTIVATORS
• Screened the LifeArc Index set (11k compounds)
• TREK-2, THIK-1, TWIK-1, TASK-3 and TASK-2 initially used
• Activity calculated relative to DMSO (high) and inhibitor (low)
• Novel activators identified for multiple channels
• Not all channels ‘activatable’
• High assay performance
• Follow up studies showed activators to be selective vs other K2Ps

DEVELOPMENT OF A NOVEL TRAAK ASSAY
• TRAAK is expressed in nociceptive DRG and TG neurons
• Identified as genetic predictor of persistent postsurgical neuropathic pain
• Developed thallium flux assay to identify novel activators of TRAAK
• Initially screened 1000+ compounds of ‘drug-like library’
• Multiple novel activators identified which are being further developed

SELECTIVITY OF K2P ACTIVATORS
• TASK-3 activator (Terbinafine) and analogues screened against representatives of the K2P superfamily
• Selectivity is complex - changes in efficacy and potency
• Compounds can be activators at one channel, inhibitors at another
• Small structural changes have profound effects

CONCLUSIONS
• Assays developed to assess ‘ligandability’ and facilitate the identification of novel activators of K2P channels
• LifeArc Index set screened and novel activators of multiple K2P channels observed
• Activators show selectivity across K2P channels but selectivity and SAR are complex
• Not all K2Ps are ‘druggable’ using assay system described